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Germ-Line Mutations in the Neurofibromatosis 2 Gene: Correlations with Disease Severity and Retinal Abnormalities

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Summary

Neurofibromatosis 2 (NF2) features bilateral vestibular schwannomas, other benign neural tumors, and cataracts. Patients in some families develop many tumors at an early age and have rapid clinical progression, whereas in other families, patients may not have symptoms until much later and vestibular schwannomas may be the only tumors. The NF2 gene has been cloned from chromosome 22q; most identified germ-line mutations result in a truncated protein and severe NF2. To look for additional mutations and clinical correlations, we used SSCP analysis to screen DNA from 32 unrelated patients. We identified 20 different mutations in 21 patients (66%): 10 nonsense mutations, 2 frameshifts, 7 splice site mutations, and 1 large in-frame deletion. Clinical information on 47 patients from the 21 families included ages at onset and at diagnosis, numbers of meningiomas, spinal and skin tumors, and presence of cataracts and retinal abnormalities. We compared clinical findings in patients with nonsense or frameshift mutations to those with splice-site mutations. When each *patient* was considered as an independent random event, the two groups differed ($P \leq .05$) for nearly every variable. Patients with nonsense or frameshift mutations were younger at onset and at diagnosis and had a higher frequency and mean number of tumors, supporting the correlation between nonsense and frameshift mutations and severe NF2. When each *family* was considered as an independent random event, statistically significant differences between the two groups were observed only for mean ages at onset and at diagnosis. A larger data set is needed to resolve these discrepancies. We observed retinal hamartomas and/or epiretinal membranes in nine patients from five families with four different nonsense mutations. This finding, which may represent a new genotype-phenotype correlation, merits further study.

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Introduction

Neurofibromatosis 2 (NF2) is an autosomal dominant disorder characterized by bilateral vestibular schwannomas, schwannomas of other central and peripheral nerves, meningiomas, and ependymomas (Martuza and Eldridge 1988; Evans et al. 1992*a*; Parry et al. 1994). In addition, ~70% of patients have cataracts of the posterior capsule of the lens (Parry et al. 1994; Ragge et al. 1995). Clinical heterogeneity is a recognized feature of NF2, which has a birth incidence of ~1/35,000 (Evans et al. 1992*b*). At least two clinical subtypes have been proposed (Eldridge et al. 1991; Evans et al. 1992*a*; Parry et al. 1994). Patients with the severe (Wishart) subtype usually have onset by age 20 years, develop many CNS tumors in addition to vestibular schwannomas, and exhibit rapid clinical progression that may lead to death by the 3d or 4th decade (Wishart 1822; Evans et al. 1992*a*; Parry et al. 1994). In contrast, patients with the mild (Gardner) subtype often do not develop symptoms until the 3d decade and have few tumors other than vestibular schwannomas and a relatively benign clinical course (Gardner and Frazier 1930; Young et al. 1970; Evans et al. 1992*a*; Parry et al. 1994). In general, the manifestations of NF2 are similar among members of a family; however, some families with both mild and severe subtypes have been described (Parry et al. 1994; Baser et al., in press). Genetic linkage studies support a single locus on chromosome 22 (Rouleau et al. 1987) with no evidence of genetic heterogeneity (Narod et al. 1992), so that the clinical heterogeneity could result from different mutations within this gene. However other explanations, including modifying genes, or gene-gene or gene-environmental interactions, are also possible.

The NF2 gene was recently isolated from chromosome 22q by positional cloning, and its protein product was identified (Trofatter et al. 1993; Rouleau et al. 1993). The protein's 595 amino acids share sequence homology with moesin, ezrin, and radixin, members of the erythrocyte band 4.1 family of proteins, which are conserved throughout mammalian species and function in a number of roles, including maintaining membrane

Table 1

Clinical Findings in 47 Patients from 21 Families with Identified Germ-Line Mutations and in 17 Patients from 11 Families with Unknown Mutations

Type and Patient[a]	Family History[b]	Age at Onset[c] (years)	Age at Diagnosis[c] (years)	Neural Tumors[d]	Cataract[e]	Retinal Changes[f]		Age at Initial Hearing Loss[g] (years)	Last Age[h] (years)	Mutation		
						Hamartoma	Epiretinal Membrane			Altered Exons[i]	Nucleotide Change	Codon Change
Nonsense and frameshift mutations:												
G17690	Y	5	10	BVS, 10M, 8Sp, 8P	Y	N	Y	AS	12	2	169C→T	Arg57→stop
Sib	Y	4	12	BVS, 0M, 1Sp, 2P	Y	Y	N	AS	13	2		
FF5863	Y	8	23	BVS, 6M, 10Sp, 0P	Y	N	Y	22	41	3	331C→T	Gln111→stop
G16221	N	5	18	BVS, 5M, >25Sp, 14P	Y	N	N	18	25	6	544C→T	Glu182→stop
G17692	N	4	15	BVS, 10M, ND, 21P	N	N	N	16	22	6	551G→A	Trp184→stop
G5145	N	12	18	BVS, 9M, >25Sp, 14P	Y	N	N	18	39*	6	586C→T	Arg196→stop
G16215	N	10	12	BVS, 6M, >25Sp, 18P	Y	N	N	11	19	10	997delC	Gln333fs345→stop
G1703[j]	Y	26	27	BVS, 5M, >16Sp, 11P	Y	N	N	28	48	11	1021C→T	Arg341→stop
Sib	Y	18	26	0VS, >9M, >14Sp, 5P	Y	N	N	27	43*	11		
G17900[k]	Y	5	16	BVS, 7M, 9Sp, 7P	Y	Y	N	15	36	11	1021C→T	Arg341→stop
Offspring	Y	4	7	BVS, 0M, 0Sp, 13P	Y	Y	N	9	9	11		
Offspring	Y	8 mo	4	BVS, 0M, 7Sp, 4P	N	Y	N	AS	6	11		
Parent	Y	5	53	UVS, 1M, 1Sp, 13P	Y	Y	N	21	68	11		
FF3053	N	15	24	BVS, 2M, ND, 0P	N	N	N	24	25	11	1030C→T	Gln344→stop
G16206	N	3	11	BVS, 2M, 9Sp, 4P	Y	N	Y	12	17	12	1198C→T	Gln400→stop
G17693	Y	14	17	BVS, 4M, >13Sp, 4P	Y	Y	Y	25	28	13	1396C→T	Arg466→stop
Parent	Y	43	53	BVS, 4M, 1Sp, 1P	N	N	N	43	55	13		
G6272[l]	Y	19	20	BVS, 1M, 4Sp, 5P	Y	N	N	22	45	14	1499delT	Leu500fs514→stop
Sib	Y	17	18	BVS, >2M, 0Sp, 11P	Y	N	N	17	38*	14		
G17696	Y	16	26	BVS, 4M, 5Sp, 4P	Y	N	N	16	36	15	1606C→T	Gln536→stop
Altered splice-acceptor sites:												
G16209	Y	6	33	BVS, 9M, 4Sp, 4P	Y	N	N	24	48	5	448-2a→g	
Offspring	Y	AS	20	UVS, 1M, 0Sp, 0P	N	N	N	AS	21	5		
G16212	N	18	25	BVS, 5M, 7Sp, 8P	Y	N	N	24	39	5	448-2a→t	
G17697	Y	28	36	BVS, 1M, 7Sp, 4P	Y	N	N	28	48	13	1341-1g→a	
Offspring	Y	16	17	BVS, 2M, 11Sp, 6P	Y	N	N	16	26*	13		
Offspring	Y	17	20	BVS, 4M, 7Sp, 0P	Y	N	N	27	27	13		
G18345	N	28	39	BVS, 2M, 2Sp, 3P	Y	N	N	39	53	15	1575-1g→a	
Altered splice-donor sites:												
G20781	N	36	45	BVS, 9M, 11Sp, 2P	Y	N	N	36	48	1	Glu38→Val	
G17695	Y	24	34	0VS*, 1M, >10Sp, 0P	N	N	N	34	40	5	516+1g→a	
Offspring	Y	AS	19	BVS, 0M, ND, 0P	N	N	N	AS	19	5		
G17302	Y	25	67	BVS, 0M, 0Sp, 1P	N	N	N	25	72	8	810+2ins3bp	
Offspring	Y	39	49	BVS, 0M, 0Sp, 0P	N	N	N	39	49	8		
Offspring	Y	17	44	BVS, 0M, 0Sp, 0P	N	N	N	17	47	8		
Offspring	Y	16	39	BVS, 0M, ND, 0P	Y	N	N	39	39	8		
Grandchild	Y	21	24	UVS, 0M, 0Sp, 0P	N	N	N	21	24	8		
Grandchild	Y	AS	16	BVS, 0M, 0Sp, 0P	N	N	N	AS	18	8		

In-frame deletion:											
G5722[m]	Y	50	70	UVS, 0M, ND, 0P	Y	N	N	50	71*	13–14	del1341-1574
Sib	Y	24	41	BVS, 0M, 1Sp, 0P	Y	N	N	24	67	13–14	
Sib	Y	40	51	BVS, 0M, ND, 0P	U	N	N	40	74	13–14	
Sib	Y	19	41	BVS, 0M, ND, 2P	Y	N	N	41	62	13–14	
Sib	Y	33	34	BVS, 1M, ND, 4P	Y	N	N	33	56*	13–14	
Nephew	Y	28	32	BVS, 1M, 0Sp, 1P	Y	N	N	28	43	13–14	
Niece	Y	AS	26	BVS, 0M, 1Sp, 8P	Y	N	N	30	38	13–14	
Nephew	Y	AS	33	BVS, 0M, 0Sp, 0P	Y	N	N	38	39	13–14	
Nephew	Y	22	22	BVS, 0M, 0Sp, 1P	Y	N	N	AS	32	13–14	
Niece	Y	AS	25	BVS, 0M, 0Sp, 0P	Y	N	N	AS	30	13–14	
Nephew	Y	AS	19	BVS, 0M, ND, 1P	Y	N	N	AS	23	13–14	
Unidentified mutations:											
G16218	N	9	19	BVS, 4M, >10Sp, >40Sp	N	N	N	19	21*	U	
G17709	Y	16	17	BVS, 0M, 0Sp, 5P	Y	N	N	18	27	U	
Sib	Y	18	20	BVS, 0M, >10Sp, 4P	Y	N	N	18	32		
Sib	Y	16	18	BVS, 0M, 0Sp, 1P	Y	N	N	16	29		
G19757[o]	N	18	19	BVS, 5M, 4Sp, 10P	Y	N	N	18	28	U	
G20782	N	22	47	BVS, 4M, ND, 1P	Y	N	N	28	57	U	
G17700	N	27	32	BVS, 7M, 4Sp, 3P	Y	N	N	32	40	U	
G17901	Y	AS	40	BVS, 0M, ND, 0P	N	N	N	AS	40	U	
FF5734	Y	20	29	BVS, 0M, ND, 0P	N	N	N	20	29	U	
G19759	Y	21	22	BVS, 0M, ND, 9P	N	N	N	30	40	U	
G19758	Y	32	39	BVS, 0M, ND, 1P	N	N	N	32	53	U	
Offspring	Y	29	31	BVS, 0M, 2Sp, 0P	N	N	N	33	34		
Sib	Y	18	23	BVS, 0M, 0Sp, 1P	N	N	N	23	45		
Niece	Y	13	13	BVS, 0M, 4Sp, 3P	N	N	N	21	23		
G17710	Y	35	44	BVS, 0M, 0Sp, 1P	Y	N	N	35	47	U	
Son	Y	19	21	BVS, 0M, 0Sp, 0P	Y	N	N	19	21		
G17707	N	49	56	UVS, 0M, 0Sp, 4P	Y	N	N	53	59	U	

[a] The first patient in each family whose DNA was analyzed by SSCP analysis is identified by the assigned laboratory specimen number. The other examined family members are designated by their relationship to this individual.

[b] Patients were considered to have a family history of NF2 if at least one first-degree relative had been given this diagnosis.

[c] In addition to symptoms from CNS tumors, we considered as initial manifestations painful or growing peripheral tumors and vision loss caused by cataracts or other ocular abnormalities. AS = asymptomatic at the time of diagnosis.

[d] The types of intracranial tumors were identified through imaging studies and/or by histopathologic examination. BVS = bilateral vestibular schwannoma; UVS = unilateral vestibular schwannoma; M = meningioma. (The asterisk [*] signals that patient G17695 had bilateral seventh cranial nerve schwannomas.) The analysis of spinal tumors was based on the total number of spinal or spinal nerve root tumors (Sp) observed on imaging studies or confirmed by histopathology; they were not subdivided by type. Most spinal tumors were schwannomas; a few patients had one or more meningiomas, ependymomas, or astrocytomas. ND = spinal imaging studies were not done. The peripheral tumors were identified through skin examination and/or histopathology; most were schwannomas or had an appearance and texture consistent with this diagnosis.

[e] Based on a dilated slit-lamp examination. Y = positive for posterior subcapsular/capsular or peripheral cortical cataract or both; N = negative; U = type of lens opacity was not known.

[f] Based on a dilated slit-lamp examination.

[g] Reported by the patient or detected on audiometry.

[h] Age at last contact or at death. Patients who have died are indicated by an asterisk (*).

[i] The exon(s) altered by the identified germ-line mutation. Each mutation is indicated only for the subject. U = mutation unknown.

[j] G17303 and his sister were patients III_5 and III_6 in the pedigree reported by Lee and Abbott (1969).

[k] G17900, his two children, and his father were reported by Bouzas et al. (1992).

[l] G6272 and her brother were part of family 9 (Narod et al. 1992).

[m] This family was reported as the FR family (Allen et al. 1974; Kaiser-Kupfer et al. 1989; Parry et al. 1991), kindred II (Kanter et al. 1980) and family 3 (Narod et al. 1992; Ruttledge et al. 1993).

[n] This individual was from the family first reported by Gardner and Frazier (1930) and Young et al. (1970).

stability by binding integral membrane proteins to the spectrin-actin cytoskeleton (Sato et al. 1992). The NF2 gene has several characteristics of a tumor suppressor: loss of the wild-type allele has been demonstrated in schwannomas and meningiomas from NF2 patients (Twist et al. 1994; Sainz et al. 1995) and mutated alleles have been found in sporadic schwannomas and meningiomas, together with loss of heterozygosity for RFLPs on chromosome 22q (Lekanne Deprez et al. 1994; Merel et al. 1995*b*).

Over 40 different germ-line mutations have been reported in unrelated NF2 patients in conjunction with clinical information (MacCollin et al. 1993; Arai et al. 1994; Bourn et al. 1994*a*, 1994*b*; Merel et al. 1995*a*; Bijlsma et al. 1995; Sainz et al. 1995; Kluwe et al. 1995; Evans et al. 1995; Kluwe and Mautner 1996). Most of these mutations are nonsense and frameshifts predicted to lead to the synthesis of a truncated protein, and patients with them usually have severe NF2 (Bourn et al. 1994*a*, 1994*b*; Merel et al. 1995*a*; Sainz et al. 1995). In contrast, the less frequent splice-site and missense mutations have occurred in both mildly and severely affected individuals (MacCollin et al. 1993; Bourn et al. 1994*b*; Merel et al. 1995*a*; Bijlsma et al. 1995; Evans et al. 1995; Kluwe and Mautner 1996) with no firm correlation being discerned between clinical subtype and location or type of the mutation.

Studies of syndromes associated with other tumor-suppressor genes as well as with the *RET* oncogene have found correlations between mutations in specific regions and a diverse array of both neoplastic and non-neoplastic findings (Olschwang et al. 1993; Caspari et al. 1994; von Heyningen 1994; Chen et al. 1995; Davies et al. 1995). Elucidation of similar genotype-phenotype correlations in NF2 might provide insight into the functional domains of the protein and be useful in counseling patients and at-risk family members. Therefore, we used SSCP analysis to search for germ-line NF2 mutations in 32 unrelated patients who had undergone a systematic evaluation including imaging and ophthalmologic and audiological studies. This report describes the 20 germ-line mutations that were identified in 21 patients and the clinical findings in these individuals and 26 of their affected relatives.

Subjects and Methods

Subjects and Clinical Evaluation

The 32 NF2 families enrolled in this study were evaluated under an approved protocol at the NIH (31 families) or on a field trip to the Geisinger Clinic (1 family) between August 1987 and February 1994. Fifteen families were referred by physicians or other health care providers, and 13 were self-referred. Three families were ascertained through a review of records of patients seen by one of us (R.E.) prior to August 1987, and one was ascertained through a report of one large family suspected to have NF2 (Lee and Abbott 1969). After obtaining informed consent, evaluation of index cases and their first-degree relatives included a physical examination with emphasis on evaluation of skin and neurologic status, a complete eye examination with slit-lamp evaluation of the lens and fundus, audiometry, auditory brainstem-evoked responses, and magnetic resonance imaging (MRI) of the brain before and after administration of gadolinium. MRI of all or part of the spine was done on all patients who had ever reported symptoms and on a subset of asymptomatic patients. Blood was obtained from patients and first-degree relatives for molecular studies. The diagnosis of NF2 was based on criteria established in 1991 (National Institutes of Health Consensus Conference Statement 1994) with modifications for sporadic cases (Evans et al. 1992*a*).

We designated the affected person from each family whose DNA was used in the mutation analysis as the subject; the subject was not always a proband. We examined 64 patients: the subject and one or more affected relatives from 13 families (n = 45 patients) and the subject only from 19 families. The clinical findings in the 32 subjects and their 32 examined relatives are given in table 1. Details of the ascertainment, clinical assessment, and clinical findings in patients from 26 of these families have been described elsewhere (Parry et al. 1994). Four of these families and one other included here (subject G19759) have also been reported separately; these citations are in the footnotes of table 1. Five subjects (FF5863, G17901, FF5734, G17710, and G17707) have not been described before.

Lymphoblast Lines and DNA and RNA Extraction

For the majority of patients, lymphoblast lines were established from peripheral blood as described elsewhere (Anderson and Gusella 1984). DNA was extracted directly from peripheral blood leukocytes or cultured lymphoblast lines by well-established methods (Jacoby et al. 1994). RNA was extracted from cultured lymphoblasts by using an acid guanidium thiocyanate isolation protocol. First-strand cDNA was synthesized from ~2 μg of total RNA using reverse transcriptase and random priming (Superscript II Reverse Transcriptase, Gibco-BRL).

Mutation Analyses

SSCP analysis was carried out by a standard approach (MacCollin et al. 1994). In brief, oligonucleotide primers were designed to amplify the 17 known exons of the NF2 gene and the adjacent splice junctions. This primer set amplified 60 bp of the 5′ UTR region and 98 bp of the 3′ UTR in addition to the entire coding sequence. All samples were run in duplicate under differing electrophoretic conditions (primarily 6% vs. 8% polyacrylamide concentration). When a known positive control

was available, it was run in parallel for each exon assayed.

Direct sequencing of both strands of PCR-amplified exons was performed as described elsewhere (Jacoby et al. 1994). In selected cases, the amplified products were also cloned in T vector (Novagen), and the DNA obtained from several clones was sequenced using a Sequenase Kit (USB). In a single case, a large deletion was detected by Southern blot analysis as described elsewhere (patient GUS5722, Trofatter et al. 1993).

Statistical Analysis

Subjects and affected relatives for whom no NF2 mutation was identified were excluded from the statistical analysis. The analysis of variance procedure, ANOVA, was used to test the null hypothesis that the mean ages for each of the age variables and the mean numbers of tumors for each tumor type were statistically equivalent for each type of mutation. These variables included age at onset of symptoms, hearing loss and diagnosis, and number of meningiomas, spinal tumors, and skin tumors. The χ^2 procedure was used to test the null hypothesis of independence between the categorical variables of clinical findings and type of mutation. The clinical parameters examined included proportions of patients with different types of tumors (meningiomas, spinal tumors, and skin tumors), hearing loss, and ocular abnormalities. Mixed models were fitted, incorporating a fixed effect for group (identified on the basis of type of mutation) and random effects for individuals and family. In the unweighted analysis, each *patient* was considered as an independent random event, whereas in the weighted analysis, each *family* was treated in this way. Two mutation groups were compared: nonsense and frameshift mutations versus splice-site mutations. One family had a large in-frame deletion whose molecular outcome was predicted to differ from that of either of these types of mutations, so we excluded it from the entire analysis.

Results

Genotypic Analyses

The coding region of the NF2 gene was scanned in the 32 unrelated subjects: 20 unique SSCP shifts were detected in 21 individuals. Alterations occurred in 12 of 17 exons; all samples gave normal patterns for exons 4, 7, 9, 16, and 17. Two unrelated subjects (G17303 and G17900) gave identical patterns in exon 11. Eleven subjects did not show variation in any exons.

The results of the sequence analysis are given in table 1. We characterized 20 different mutations: 16 were single nucleotide substitutions (10 nonsense, 4 splice-acceptor sites, 2 splice-donor sites), 3 were deletions, and 1 was an insertion. The A-to-T transition at nt 114-1 was predicted to produce the missense mutation Glu38Val and also to alter the exonic portion of the splice-donor consensus sequence. Sequence analysis of cDNA from the mutated allele revealed an insertion of 56 bp between exons 1 and 2. This sequence corresponded to the first 56 bp of intron 1 (accession number L27131; Jacoby et al. 1994) and included an in-frame stop codon at 114+31. Two of the deletions were frameshifts; the third deletion removed nt 1341–1574 of the cDNA, which corresponded to exons 13 and 14 but left the reading frame intact. Finally, a 3-bp insertion resulted in a splice-donor mutation. One nonsense mutation, a C-to-T transition at nt 1021, was detected in two unrelated subjects. The mutations in subjects G16209, G16212, G16221, G16215, G6272, and G5722 have been reported before (Trofatter et al. 1993; MacCollin et al. 1994).

Segregation of Alterations within NF2 Families

We confirmed the segregation of variants within NF2 families using SSCP analysis, restriction enzyme digest, or, in the case of G5722, Southern blotting. Lymphocyte DNA was available on both parents of seven sporadic subjects; none of the parents showed the aberrant patterns seen in their affected offspring. We also assayed lymphocyte DNA from 14 affected relatives of eight subjects and confirmed that, within each family, the subject and affected relative(s) carried the same mutant allele. In one of these families, DNA from subject G17690 and his affected brother produced the same SSCP pattern in exon 2, which was not seen in DNA from either clinically normal parent. Microsatellite analysis ruled out nonpaternity, leading to the suspicion that the mutation was present in a subpopulation of germ cells in one of the parents.

Correlation between Genotype and Phenotype

The clinical findings in patients from families with identified germ-line mutations are given in table 1. To determine whether there were statistically significant clinical differences between patients with the different types of mutations, we compared the findings in patients with nonsense or frameshift mutations with those in patients with splice-site mutations (table 2). As noted in Subjects and Methods, we excluded the family with the in-frame deletion from the analysis because this was a novel mutation. In the unweighted analysis there were statistically significant differences ($P \leq .05$) for every variable except for proportions of patients with hearing loss ($P = .76$), cataracts ($P = .12$), and epiretinal membranes ($P = .06$). The patients with nonsense or frameshift mutations had much younger mean ages at onset of symptoms (including hearing loss) and at diagnosis than did patients with splice-site mutations. The proportion of patients with each type of tumor was at least 1.3-fold higher in the group with nonsense or frameshift mutations than in the group with splice-site mutations, and the patients with nonsense or frameshift

Table 2

Analysis of Clinical Findings in NF2 Patients with Nonsense or Frameshift Mutations Versus Splice-Site Mutations

Feature	Nonsense or Frameshift Mutations	Splice-Site Mutations	All Patients[b]	*P* Value, Unweighted Analysis[b]	*P* Value, Weighted Analysis[c]
Families (*n*)	13	7	20	ND	ND
Patients (*n*)	20	16	36	ND	ND
Mean age at onset (years)	11.7	22.4	15.9	.002	.02
Mean age at diagnosis (years)	20.5	32.9	26.0	.009	.03
Percent with hearing loss	85	81.3	83.3	.76	.64
Mean age at first hearing loss (years)	20.2	28.4	23.8	.01	.01
Percent with meningiomas (mean no. of tumors)	85 (4.4)	50.0 (2.1)	69.4 (3.3)	.02 (.04)	.21 (.34)
Percent with spinal tumors (mean no. of tumors)	88.9[d] (9.6)	57.1[e] (4.2)	75.0[f] (7.3)	.04 (.04)	.38 (.14)
Percent with skin tumors (mean no. of tumors)	90 (8.0)	43.8 (1.8)	69.4 (5.2)	.003 (ND)	.25 (ND)
Percent with cataracts	80	56.3	69.4	.12	.62
Percent with retinal harmartomas	30	0	16.7	.02	.27
Percent with epiretinal membranes	20	0	11.1	.06	.16
Percent with retinal hamartomas and/or epiretinal membranes	45	0	25.0	.002	.08

NOTE.—For comparison, the following are the clinical findings in the 11 patients from the family with the in-frame deletion: mean age at onset = 30.9 years; mean age at diagnosis = 35.8 years; percent with hearing loss = 72.7; mean age at first hearing loss = 35.5 years; percent with meningiomas = 9.1; mean no. of meningiomas = 0.2; percent with spinal tumors = 33.3; mean no. of spinal tumors = 0.3; percent with skin tumors = 45.5; mean no. of skin tumors = 1.5; percent with cataracts = 100.0; percent with retinal hamartomas and/or epiretinal membranes = 0.

[a] Total no. of patients with nonsense, frameshift, or splice-site mutations.

[b] In this analysis, each patient was considered to be a random, independent event. ND = analysis not done.

[c] In this analysis, each family was considered to be a random, independent event; if a family had *n* examined patients, the clinical findings in each patient contributed 1/n to the total for the family. ND = analysis not done.

[d] Total no. of patients on whom information was available = 18.

[e] Total no. of patients on whom information was available = 14.

[f] Total no. of patients on whom information was available = 32.

mutations had a higher mean number of each type of tumor. Retinal hamartomas were documented only among patients with nonsense or frameshift mutations ($P = .02$). When the analysis was repeated with each family being assumed to represent a random independent event, statistically significant differences ($P \leq .05$) were observed only for mean ages at onset, diagnosis, and hearing loss.

The nine patients with retinal hamartomas, epiretinal membranes, or both were from a subset of five families with nonsense mutations (table 1). When we created a new variable (retinal hamartomas and/or epiretinal membranes) to reflect the conjoint occurrence of these findings and used it to compare the data between the two mutation groups, the difference was statistically significant in the unweighted analysis ($P = .002$) and nearly so in the weighted analysis ($P = .08$) (table 2). Retinal hamartomas were reported in one other family with a nonsense mutation, in two deceased nieces of subject G17303. Since we did not detect retinal abnormalities in either subject G17303 or his sister (table 1), in the analysis we did not count this family as having retinal hamartomas.

Clinical Heterogeneity within Families

We examined at least two patients from 10 of these families (table 1). There were four families in which either sibs of similar ages had markedly different clinical findings or a parent had fewer or more-mild findings than their offspring: subject G17690 had many more CNS and peripheral tumors than his brother, who was 2 years older; subject G17900 had more CNS tumors than his father, who was 32 years older; age at onset of symptoms in the mother of subject G17693 was almost

30 years older than in her daughter, and she had fewer spinal tumors; and age at onset of symptoms in subject G17697 was more than 12 years older than in his two affected children, and he had fewer intracranial meningiomas. The young ages at diagnosis of some relatives of subjects G17900, G16209, G17695, G17302, G17697, and G5722 are the result of clinical screening of at-risk family members.

Subjects with No Identifiable Mutation After SSCP Analysis

No germ-line mutations were detected in subjects from 11 families (table 1). None of these patients had retinal hamartomas or epiretinal membranes. Subject G16218 had two clinical findings that were not present in any other patient in this study: he had >40 dermal schwannomas removed, and at age 21 he developed a rapidly progressive hypertrophic generalized peripheral neuropathy that was thought to be a manifestation of NF2 (Mori et al. 1985; Thomas et al. 1990; Kilpatrick et al. 1992; Evans et al. 1992*a*; Parry et al. 1994); it resulted in respiratory failure which caused his death.

Discussion

In this study we used an arbitrary set of easily categorized or quantified clinical variables to examine disease status in 47 NF2 patients from 21 families with 20 different germ-line mutations. Our results demonstrated that patients with nonsense or frameshift mutations had more manifestations compatible with severe disease than patients with splice-site mutations: their findings included earlier mean ages at onset of symptoms, including hearing loss, and diagnosis, and a higher frequency and mean number of meningiomas and spinal and peripheral tumors. However, when we used the average values of the clinical parameters for each family in the analysis, the statistically significant differences between the two mutation groups were limited to the three age variables. This suggests that intrafamilial variability in the frequency and number of neural tumors was sufficient to decrease the strength of the correlation between genotype and phenotype. With regard to ocular findings, there was no association between type of mutation and frequency of cataracts; however, retinal hamartomas and epiretinal membranes occurred only in patients from a few families with nonsense mutations. If the latter observation is confirmed in additional NF2 families, it will explain the association between retinal hamartomas and young age at onset of first symptoms (Parry et al. 1994; Ragge et al. 1995) and an increased frequency of CNS and peripheral neural tumors (Parry et al. 1994) and support a common pathogenesis for the two types of retinal abnormalities (Schachat et al. 1984; Font et al. 1989).

These results confirm the reported association between nonsense and frameshift mutations and clinical findings considered consistent with severe disease (Bourn et al. 1994*b*; Merel et al. 1995*a*). The 10 nonsense mutations in our patients included three (in codons 57, 111, and 341) that had been identified previously in up to six unrelated patients (Bourn et al. 1994*b*; Merel et al. 1995*a*; Sainz et al. 1995) and seven reported for the first time in conjunction with clinical information. Two additional nonsense mutations, involving codons 262 and 463, have been described in other unrelated NF2 patients (Merel at al. 1995*a*; Sainz et al. 1995). The nonsense mutations in codons 57, 198, 262, 341, and 466 are C-to-T transitions at the site of a CpG dinucleotide in an arginine-specifying CGA codon. These five codons appear to be hotspots for germ-line and/or somatic NF2 mutations (Bourn et al. 1994*b*; Sainz et al. 1995). We also identified two unique frameshift mutations, which brings to 19 the number of different frameshifts that have been reported in clinically described NF2 patients (Bourn et al. 1994*b*; Merel et al. 1995*a*). All 53 NF2 patients from the 45 different families with these two types of mutations had either "severe" NF2 (Bourn et al. 1994*b*) or developed symptoms at a young age (usually <25 years) and had at least one intracranial meningioma and/or spinal tumor.

In our series, six nonsense mutations truncated the NF2 protein within the first 342 residues, the region of greatest homology with moesin, ezrin, and radixin (Trofatter et al. 1993). This includes the N-terminal domain, thought to bind to proteins in the cell membrane, and a portion of the alpha helical structure that may attach to components of the cytoskeleton. The function of the highly charged and hydrophilic C-terminal domain is unknown; this region has less sequence homology but is present to some degree in all related proteins. Our most 3′ truncating mutation introduced a stop at codon 536; it demonstrates conclusively that removing the last 59 amino acids from the C-terminus impairs the protein's function and leads to significant disease.

We identified seven putative splice-site mutations. The mutations involving exons 1, 13, and 15 (the latter two exons have $3n + 1$ bases) should lead to an mRNA containing a premature stop codon and produce a truncated protein. The other four mutations involving exons 5 and 8 (each with $3n$ bases) are predicted to remove the respective exon from the resulting mRNA transcript. We have confirmed the expected molecular outcome only for the exon 1 mutation. In numerous experiments with lymphoblast cell lines from patients with the other six mutations, NF2 gene expression was not sufficiently robust to confirm the presence of abnormally spliced transcripts (M. MacCollin, unpublished data). However, several lines of evidence suggest that all seven mutations are disease-related and not benign polymorphisms. First of all, they were the only mutations

detected in the 17 NF2 exons examined in these seven subjects. Second, we did not find any of these mutations when we screened chromosome 22 DNA from >150 normal individuals. Finally, we did not find the mutations identified in G16209 and G16212, who were both sporadic cases, in either set of unaffected parents; we did detect the mutations identified in G16209, G17302, and G17697 in at least one affected relative, and the mutation in subject G17695 has been reported in one other presumably unrelated NF2 patient (Merel et al. 1995*a*).

As a group, the patients with these seven splice-site mutations presented at a later age and had a lower frequency and mean number of meningiomas and other neural tumors than the patients with nonsense or frameshift mutations. Variable amounts of normal splicing may explain the milder phenotypes associated with these mutations. Our data also suggest that similar types of splice-site mutations can have different clinical outcomes. Among patients with the three mutations predicted to truncate the C-terminus, only two from the family with the exon 13 mutations had both early ages at onset of symptoms and multiple meningiomas and spinal tumors. Among patients with the three mutations predicted to delete exon 5 from the mRNA, only those with altered splice-acceptor sites had both early ages at onset and at least one meningioma. A similar dichotomy between "severe" splice-acceptor and "mild" splice-donor mutations has been reported for mutations in exon 5 as well as exon 2 (Merel et al. 1995*a*); it was the only genotype-phenotype correlation observed among patients with nine different splice-site mutations. Finally, none of the patients in the family with the exon 8 splice donor mutation had meningiomas or spinal tumors. The very mild phenotype in this family and in the family with the in-frame deletion may result from the residual activity of an NF2 protein that has intact N- and C-terminal domains.

Previous reports of genotype-phenotype correlations either did not provide information on eye findings (Bourn et al. 1994*b*), or it was limited in scope and not available on all patients (Merel et al. 1995*a*). Cataracts, the most common ocular manifestation of NF2 (Bouzas et al. 1993; Ragge et al. 1995) were seen in 35 of 46 (76%) of our patients with identified germ-line mutations and in at least one affected person from 18 of the 21 families (table 1). There was no difference in the frequency of cataracts between patients with nonsense or frameshift versus splice-site mutations. However, among patients from the two large families with relatively mild clinical findings, those with the exon 8 splice-site mutation (subject G17302 and his relatives) had a lower frequency of cataracts (1 [16.7%] of 6 patients) than subject G5722 and his relatives with the large in-frame deletion (10 [100%] of 10) (table 1). The frequency of some cataracts in NF2 increases with age (Bouzas et al. 1993); however, this difference is not age related, because the mean age at last eye examination of the five patients who did not have cataracts (41.6 years) did not differ from the mean age at diagnosis of cataract in their one relative (39 years) or in the 10 patients with the in-frame deletion (40.7 years). Because this observation is based on a small number of patients from two families with novel mutations, the question of the variable expression of cataracts needs to be examined in a larger data set.

Retinal hamartomas and epiretinal membranes are part of a wide array of ocular abnormalities that occur in NF2 (Cotlier 1977; Landau et al. 1990; Good et al. 1991; Sivalingham et al. 1991; Bouzas et al. 1992; Kaye et al. 1992; Landau and Yasargil 1993; Ragge et al. 1995). Both retinal lesions are thought to result from developmental defects that involve glial cells and the vascular epithelium; consequently, it has been suggested that they represent different aspects of the same pathological process (Schachat et al. 1984; Font et al. 1989). Our observations of retinal hamartomas and epiretinal membranes occurring separately in sibs in one family and concomitantly in one patient from a second family (table 1) support a common pathogenesis. The nine patients with retinal abnormalities were from five families with nonsense mutations. Retinal hamartomas were also reported in a sixth family in our study with a nonsense mutation. Two of these six families had the same nonsense mutation in codon 341. This mutation was also present in the only other reported NF2 patient with a known germ-line mutation and retinal hamartomas (Sainz et al. 1995). The five nonsense mutations associated with retinal abnormalities were located throughout the coding region. That no other patients with nonsense or frameshift mutations had retinal hamartomas and/or epiretinal membranes could be a consequence of the decreased expression of these traits and the small number of patients in each family. Alternatively, these retinal abnormalities might occur only in a subset of truncating mutations, or they might result from other genetic or nongenetic influences. Dilated slit-lamp examinations should be part of the routine care of all NF2 patients; it is hoped that future reports of patients with germ-line mutations will describe ocular findings so that their frequency and distribution can be determined.

Our results are based on data that have some limitations. Most likely, the 32 unrelated patients screened for germ-line mutations do not represent the full range or relative proportions of NF2 phenotypes. Because our ascertainment relied primarily on physician referral and self-referral, our patients tended to be severely affected. Many were also sporadic cases, which limited our ability to examine intrafamilial clinical variability. In contrast, a few large families ascertained for linkage analysis had mild disease. We identified germ-line mutations in 66% (21/32) of these families. However, we found mutations

in only 5 (36%) of 14 families with clinical findings at the mild end of the spectrum, compared with 16 of 18 families with predominantly severe NF2 (89%). An important question is the location of the germ-line mutations in the 11 remaining families. These mutations may be in unscreened regions of the introns and the 3′ UTR and 5′ regulatory elements. We may have also missed large intragenic deletions that removed regions encompassed by our PCR primers, as well as deletions that included the entire gene (Sanson et al. 1993; Watson et al. 1993). However, our present screen did identify an in-frame deletion that removed two exons. This mutation was found initially by Southern blot analysis (Trofatter et al. 1993).

Our results have confirmed the association between nonsense and frameshift mutations and some clinical manifestations compatible with severe disease and have demonstrated a possible correlation between specific retinal abnormalities and some nonsense mutations. In addition, our data and other reports of intrafamilial variability in the clinical expression of NF2 (Baser et al., in press) raise questions regarding factors other than the intrinsic property of individual mutations that might influence phenotype. Future molecular studies of clinically well-described patients and families may identify additional genotype-phenotype correlations and provide new clues to the functional domains of the protein, the possible tissue-specific action of the NF2 gene, and the role of other genes or environmental processes in modifying its expression.

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References

Allen JC, Eldridge R, Young D (1974) Early-onset acoustic neuroma: genetic, clinical and nosologic aspects. Birth Defects 10:171–184

Anderson M, Gusella J (1984) Use of cyclosporin A in establishing Epstein-Barr virus-transformed human lymphoblastoid cell lines. In Vitro 20:856–858

Arai E, Ikeuchi T, Nakamura Y (1994) Characterization of the translocation breakpoint on chromosome 22q12.2 in a patient with neurofibromatosis 2 (NF2). Hum Mol Genet 6:937–939

Baser ME, Mautner VF, Ragge NK, Nechiporuk A, Riccardi VM, Klein J, Sainz J, et al. Presymptomatic diagnosis of neurofibromatosis 2 using linked genetic markers, neuroimaging and ocular examinations. Neurology (in press)

Bijlsma EK, Merel P, Fleury P, van Asperen CJ, Westerveld A, Delattre O, Thomas G, et al (1995) Family with neurofibromatosis type 2 and autosomal dominant hearing loss: identification of carriers of the mutated NF2 gene. Hum Genet 96:1–5

Bourn D, Carter SA, Evans DGR, Goodship J, Coakham H, Strachan T (1994*a*) A mutation in the neurofibromatosis type 2 tumor-suppressor gene, giving rise to widely different clinical phenotypes in two unrelated individuals. Am J Hum Genet 55:69–73

Bourn D, Carter SA, Mason S, Evans DGR, Strachan T (1994*b*) Germline mutations in the neurofibromatosis type 2 tumour suppressor gene. Hum Mol Genet 5:813–816

Bouzas EA, Parry DM, Eldridge R, Kaiser-Kupfer MI (1992) Familial occurrence of combined pigment epithelial and retinal hamartomas associated with neurofibromatosis 2. Retina 12:103–107

——— (1993) Visual impairment in patients with neurofibromatosis 2. Neurology 43:622–623

Caspari R, Friedl W, Mandl M, Moslein G, Kadmon M, Knapp M, Jacobasch K-H, et al (1994) Familial adenomatous polyposis: mutation at codon 1309 and early onset cancer. Lancet 343:629–632

Chen F, Kishida T, Yao M, Hustad T, Glavac D, Dean M, Gnarra JR, et al (1995) Germline mutations in the von Hippel-Lindau disease tumor suppressor gene: correlations with phenotype. Hum Mutat 5:66–75

Cotlier E (1977) Cafe-au-lait spots of the fundus in neurofibromatosis. Arch Ophthalmol 95:1990–1992

Davies DR, Armstrong JG, Thakker N, Horner K, Guy SP, Clancy T, Sloan P, et al (1995) Severe Gardner syndrome in families with mutations restricted to a specific region of the *APC* gene. Am J Hum Genet 57:1151–1158

Eldridge R, Parry DM, Kaiser-Kupfer MI (1991) Neurofibromatosis 2: clinical heterogeneity and natural history in 39 individuals in 9 families and 16 sporadic cases. Am J Hum Genet Suppl 49:A676

Evans DGR, Bourn D, Wallace A, Ramsden RT, Mitchell JD, Strachan T (1995) Diagnostic issues in a family with late onset type 2 neurofibromatosis. J Med Genet 32:470–474

Evans DGR, Huson SM, Donnai D, Neary W, Blair V, Newton V, Harris R (1992*a*) A clinical study of neurofibromatosis 2. Q J Med 84:603–18

Evans DGR, Huson SM, Donnai D, Neary W, Blair V, Teare D, Newton V, et al (1992*b*) A genetic study of type 2 neurofibromatosis in the United Kingdom. I. Prevalence, mutation rate, fitness, and confirmation of maternal transmission effect on severity. J Med Genet 29:841–846

Font RL, Moura RA, Shetlar DJ, Martinez JA, McPherson AR (1989) Combined hamartomas of sensory retina and retinal pigment epithelium. Retina 9:302–311

Gardner WJ, Frazier CH (1930) Bilateral acoustic neurofibromatosis: a clinical study and field survey of a family of five generations with bilateral deafness in thirty-eight members. Arch Neurol Psychiatry 23:266–302

Good WV, Brodsky MC, Edwards MS, Hoyt WF (1991) Bilateral retinal hamartomas in neurofibromatosis type 2. Br J Ophthalmol 75:190

Jacoby LB, MacCollin M, Louis DN, Mohney T, Rubio M-P, Pulaski K, Trofatter JA, et al (1994) Exon scanning for mutation of the NF2 gene in schwannomas. Hum Mol Genet 3: 413–419

Kaiser-Kupfer MI, Freidlin V, Datiles MB, Edwards PA, Sherman JL, Parry DM, McCain LM, et al (1989) The association of posterior capsular lens opacities with bilateral acoustic neuromas in patients with neurofibromatosis type 2. Arch Ophthalmol 107:541–544

Kanter WR, Eldridge R, Fabricant R, Allen JC, Koerber T (1980) Central neurofibromatosis with bilateral acoustic neuroma: Genetic, clinical and biochemical distinctions from peripheral neurofibromatosis. Neurology 30:851–859

Kaye LD, Rothner AD, Beauchamp GR, Meyers SM, Estes ML (1992) Ocular findings associated with neurofibromatosis type II. Ophthalmology 99:1424–1429

Kilpatrick TJ, Hjorth RJ, Gonzales MF (1992) A case of neurofibromatosis 2 presenting with a mononeuritis multiplex. J Neurol Neurosurg Psychiatr 52:391–393

Kluwe L, Mautner VF (1996) A missense mutation in the NF2 gene results in moderate and mild clinical phenotypes of neurofibromatosis 2. Hum Genet 97:224–227

Kluwe L, Pulst SM, Koppen J (1995) A 163-bp deletion in the neurofibromatosis 2 (NF2) gene associated with variant phenotypes. Hum Genet 95:443–446

Landau K, Dossetor FM, Hoyt WF, Muci-Mendoza F (1990) Retinal hamartoma in neurofibromatosis type 2. Arch Ophthalmol 108:328–329

Landau K, Yasargil GM (1993) Ocular fundus in neurofibromatosis type 2. Br J Ophthalmol 77:646–649

Lee DK, Abbott ML (1969) Familial central nervous system neoplasia: case report of a family with von Recklinghausen's neurofibromatosis. Arch Neurol 20:154–160

Lekanne Deprez RH, Bianchi AB, Groen NA, Seizinger BR, Hagemeijer A, van Drunen E, Bootsma D, et al (1994) Frequent NF2 gene transcript mutations in sporadic meningiomas and vestibular schwannomas. Am J Hum Genet 54: 1022–1029

MacCollin M, Mohney T, Trofatter J, Wertelecki W, Ramesh V, Gusella J (1993) DNA diagnosis of neurofibromatosis 2. JAMA 270:2316–2320

MacCollin M, Ramesh V, Jacoby LB, Louis DN, Rubio M-P, Pulaski K, Trofatter JA, et al (1994) Mutational analysis of patients with neurofibromatosis 2. Am J Hum Genet 55: 314–320

Martuza RL, Eldridge R (1988) Neurofibromatosis 2 (bilateral acoustic neurofibromatosis). N Engl J Med 318:684–688

Merel P, Hoang-Xuan K, Sanson M, Bijlsma E, Rouleau G, Laurent-Puig P, Pulst S, et al (1995*a*) Screening for germline mutations in the NF2 gene. Genes Chromosom Cancer 12:117–127

Merel P, Hoang-Xuan K, Sanson M, Moreau-Aubry A, Bijlsma EK, Lazaro C, Moisan JP, et al (1995*b*) Predominant occurrence of somatic mutations of the NF2 gene in meningiomas and schwannomas. Genes Chromosom Cancer 13:211–216

Mori M, Morisaki S, Hazama R, Tsujihata M, Nagataki S, Yonekura M, Moriyama T, et al (1985) A family of von Recklinghausen's neurofibromatosis complicated by mononeuritis multiplex, bilateral acoustic neurinomas, and falx and spinal meningiomas. No To Shinkei 37:403–408

Narod SA, Parry DM, Parboosingh J, Lenoir GM, Ruttledge M, Fischer G, Eldridge R, et al (1992) Neurofibromatosis type 2 appears to be a genetically homogeneous disease. Am J Hum Genet 51:486–496

National Institutes of Health Consensus Development Conference Statement on Acoustic Neuroma, December 11–13, 1991 (1994) Arch Neurol 51:210–207

Olschwang S, Tiret A, Laurent-Puig P, Muleris M, Parc R, Thomas G (1993) Restriction of ocular fundus lesions to a specific subgroup of APC mutations in adenomatous polyposis coli patients. Cell 75:959–968

Parry DM, Eldridge R, Kaiser-Kupfer MI, Bouzas EA, Pikus A, Patronas N (1994) Neurofibromatosis 2 (NF2): clinical characteristics of 63 affected individuals and clinical evidence for heterogeneity. Am J Med Genet 52: 450–461

Parry DM, Kaiser-Kupfer MI, Sherman JL, Pikus A, Eldridge R (1991) Neurofibromatosis 2 (bilateral acoustic or central neurofibromatosis), a treatable cause of deafness: recommendations for screening and follow-up based on the study of one large kindred. Ann NY Acad Sci 630:305–307

Ragge NK, Baser ME, Klein J, Nechiporuk A, Sainz J, Pulst S-M, Riccardi V (1995) Ocular abnormalities in neurofibromatosis 2. Am J Ophthalmol 120:634–641

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, et al (1993) Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. Nature 363:515–521

Rouleau GA, Wertelecki W, Haines JL, Hobbs WJ, Trofatter JA, Seizinger BR, Martuza RL, et al (1987) Genetic linkage of bilateral acoustic neurofibromatosis to a DNA marker on chromosome 22. Nature 329:246–248

Ruttledge MH, Narod SA, Dumanski JP, Parry DM, Eldridge R, Wertelecki W, Parboosingh J, et al (1993) Presymptomatic diagnosis for neurofibromatosis 2 with chromosome 22 markers. Neurology 43:1753–1860

Sainz J, Figueroa K, Baser ME, Mautner V-F, Pulst S-M (1995) High frequency of nonsense mutations in the NF2 gene caused by C to T transitions in five CGA codons. Hum Mol Genet 4: 137–139

Sanson M, Marineau C, Desmaze C Lutchman M, Ruttledge M, Baron C, Narod S, et al (1993) Germline deletion in a neurofibromatosis 2 type kindred inactivates the NF2 gene and a candidate meningioma locus. Hum Mol Genet 2: 1215–1220

Sato N, Funayama N, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S (1992) A gene family consisting of ezrin, radixin and moesin: its specific localization at actin filament/plasma membrane association sites. J Cell Sci 103:131–143

Schachat AP, Shields JA, Fine SL, Sanborn GE, Weingeist TA, Valenzuela RE, Brucker AJ (1984) Combined hamartomas of the retina and retinal pigment epithelium. Ophthalmology 91:1609–1615

Sivalingam A, Augsburger J, Perilongo G, Zimmerman R, Barabas G (1991) Combined hamartoma of the retina and retinal pigment epithelium in a patient with neurofibromatosis type 2. J Pediatr Ophthalmol Strabismus 28:320–322

Thomas PK, King RHM, Chiang TR, Scaravilli F, Sharma AK,

Downie AW (1990) Neurofibromatosis neuropathy. Muscle Nerve 13:93–101

Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao, MP, Parry DM, Eldridge R, et al (1993) A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 72:791–800

Twist EC, Ruttledge MH, Rousseau M, Sanson M, Papi L, Merel P, Delattre O, et al (1994) The neurofibromatosis type 2 gene is inactivated in schwannomas. Hum Mol Genet 3:147–151

von Heyningen V (1994) One gene—four syndromes. Nature 367:319–320

Watson CJ, Gaunt L, Evans G, Patel K, Harris R, Strachan T (1993) A disease-associated germline deletion maps the type 2 neurofibromatosis (NF2) gene between the Ewing sarcoma region and the leukaemia inhibitory factor locus. Hum Mol Genet 2:701–704

Wishart JH (1822) Case of tumors in the skull, dura mater, and brain. Edinburgh Med Surg J 18:393–397

Young DF, Eldridge R, Gardner WJ (1970) Bilateral acoustic neuroma in a large kindred. JAMA 214:347–353